fragment, a Fab region of an antibody, an antibody-drug conjugate, a fusion protein, a protein pharmaceutical product or a drug. In yet another aspect, an enzyme of the enzymatic digestion solution in the system of the present application is trypsin.

[0011] In one aspect, the mass spectrometer in the system of the present application is an electrospray ionization mass spectrometer, nano-electrospray ionization mass spectrometer, or a triple quadrupole mass spectrometer, wherein the mass spectrometer is coupled to a liquid chromatography system. In another aspect, the mass spectrometer in the system of the present application is capable of performing LC-MS or a LC-MRM-MS analyses.

[0012] These, and other, aspects of the invention will be better appreciated and understood when considered in conjunction with the following description and the accompanying drawings. The following description, while indicating various embodiments and numerous specific details thereof, is given by way of illustration and not of limitation. Many substitutions, modifications, additions, or rearrangements may be made within the scope of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0014] FIG. **1** shows a general workflow of using ProteoMiner™ beads to enrich HCPs including the steps of loading, washing, eluting, tryptic digesting and LC-MS/MS analysis according to an exemplary embodiment.

[0015] FIG. **2**A shows PRM signal changes of one HCP peptide, for example, IYVASVHQDLSDDDIK, derived from Poly-binding-splicing Factor PUF60 according to an exemplary embodiment.

[0016] FIG. **2**B shows PRM signal changes of one HCP peptide, for example, TYFLKPSK, derived from Protein NipSnap homolog **3**B according to an exemplary embodiment.

[0017] FIG. **3**A shows the evaluation of the reproducibility of the HCP enrichment method of the present application using a triplicate experiment according to an exemplary embodiment.

[0018] FIG. **3**B shows the results of Pearson correlation analysis to evaluate the reproducibility of the HCP enrichment method of the present application according to an exemplary embodiment.

[0019] FIG. **4** shows the results of using the HCP enrichment method of the present application to identify HCPs using NISTmAb in comparing to the HCPs identified by Doneanu et al. and Huang et al. according to an exemplary embodiment.

[0020] FIG. **5**A shows the results of detecting and identifying HCPs in samples containing mAb3 and 13 spiked-in HCPs by comparing the filtration (filter) and ProteoMiner™ methods according to an exemplary embodiment.

[0021] FIG. **5**B shows the results of detecting and identifying HCPs in samples containing mAb4 and HCP impurities by comparing the filtration (filter) and ProteoMiner™ methods according to an exemplary embodiment.

[0022] FIG. **6** shows the results of testing repeatability of the ProteoMiner™ method of the present application for HCP enrichment using samples containing mAb4 and HCP impurities according to an exemplary embodiment.

[0023] FIG. **7** shows efficiencies of native digestion and ProteoMiner™ methods for HCP enrichments using samples containing mAb5 and HCP impurities according to an exemplary embodiment.

[0024] FIG. **8** shows the comparison of native digestion and ProteoMiner™ methods for the efficiencies of HCP enrichments according to an exemplary embodiments.

DETAILED DESCRIPTION

[0025] In order to manufacture biopharmaceutical products, it is important to obtain biopharmaceutical products having high purity, since residual HCPs can compromise product safety and stability. For producing cell-based recombinant therapeutic antibodies, typically, immuno-assays, such as enzyme-linked immunosorbent assays (ELISA), have been used to monitor HCP removal (clearance) using polyclonal anti-HCP antibodies during process development. ELISA can provide semi-quantitation of total HCPs levels in high throughput. However, since polyclonal anti-HCP antibodies are used for ELISA to capture, detect and quantify total HCPs, they may not be effective in quantitating individual HCPs. In particular, some non-immunogenic or weakly-immunogenic HCPs may not be detected using ELISA.

[0026] In order to both identify and quantify HCPs, several complementary approaches have been used to monitor HCPs, such as one-dimensional/two-dimensional (1D/2D) PAGE or liquid chromatography (LC) coupled tandem mass spectrometry (LC-MS/MS). However, the wide dynamic concentration ranges of HCPs in the presence of high concentration of purified antibodies may be a major challenge for developing LC-MS methods to monitor the removal of HCP impurities. Mass spectrometry (MS) alone lacks the capability to detect low abundance targets, such as low ppm levels of HCPs, in the presence of high concentrations of therapeutic antibodies due to the wide dynamic concentration ranges, which can be over six orders of magnitude. To overcome this issue, one strategy can be to resolve the co-eluting peptides before MS analysis by adding another dimension of separation, such as 2D-LC and/or ion mobility, in combination with the data-dependent acquisition or data-independent acquisition to increase the separation efficiency.

[0027] Huang et al. (Huang et al., A Novel Sample Preparation for Shotgun Proteomics Characterization of HCPs in Antibodies, Anal Chem. 2017, May 16; 89 (10):5436-5444) describes a sample preparation method using trypsin digestion for shotgun proteomics characterization of HCP impurities in an antibody sample. Huang's sample preparation method maintains the antibody nearly intact while HCPs are digested. Huang's approach can reduce the dynamic range for HCP detection using mass spectrometry by one to two orders of magnitude compared to traditional trypsin digestion sample preparation. As demonstrated by HCP spiking experiments, Huang's approach can detect 0.5 ppm of HCPs with molecular weight greater than 60 kDa, such as rPLBL2. For example, sixty mouse HCP impurities were detected in RM 8670 (NISTmAb, NIST monoclonal antibody standard, expressed in a murine cell line, obtained from the National Institute of Standards and Technology, Gaithersburg, Md.) using Huang's approach.